DESIGN AND CONSTRUCTION OF SLIDE BASED ELECTROPORATOR FOR RESEARCH APPLICATIONS

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology

In

Biomedical Engineering

By

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May, 2014

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CERTIFICATE

This is to confirm that the Thesis entitled "**Design and Construction of Slide Based Electroporator for Research Applications"** by **M.Venkateswara Rao (212BM1429)** submitted to the National Institute of Technology, Rourkela for the honour of Master of Technology in Bio-Medical building during the session 2012-2014 is a record of Bonafide research work did by him in the Department of Biotechnology and Medical Engineering under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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ABSTRACT

Electroporation is a remarkable development in the permeability and electrical conductivity of cell plasma membrane effected by an externally applied electrical field. This technique is widely used for genetic transfection protocol of a wide array of cells for research as well as commercial applications. Though a large number of Electroporation devices are in market, they are expensive and one has to go for cumbersome work flow for transfection of cells. In the current project, a miniature slide based Electroporator was designed and fabricated to minimize the cost and work flow during cell transfection protocols in laboratories. Briefly, a circular well (25 mm diameter and 14 mm depth) was fabricated by exclusion in a thick glass slide for housing live cells in suspension. The well was connected to copper electrodes for supplying electrical pulse of high voltage and of low duration. A pulse generator was developed where a train of low amplitude pulses were generated using 555 Timer and stabilized by 7805 Regulator. A Microcontroller, 89C52 was used to control the number of pulses. A transformer with 1:100 turns ratio was used to amplify a pulse with amplitude of 9V to 700-900 V, necessary for cell membrane poration without damaging the live cells. The pulse generation system was connected to the copper electrodes in contact with the cell suspension in glass well. The Electroporation system could generate pulses with maximum amplitude of 782V and a maximum 20,000 number of pulses per second. Thus, a pulse with duration of 50 µs could be generated with the system which is highly effective for pore formation in the cell membrane without killing the cells. Further, the potentiometer used was able to vary the voltage from 50 V to 782 V while the pulse duration was varied from 50 µs to 1000 ms. Such a wide range of variations in pulse amplitude and duration equips the Electroporator to be applicable for transforming different cell lineages with molecules of different sizes.

Keywords: Electroporation, Potentiometer, Transformer Device, Pore Formation, Transfection

Acknowledgement

I express my deep sense of gratitude and indebtness to **Dr.Bibhukalyan Prasad Nayak.**, my research guide, who apart from dedicating an important part of his time, has provided me with a logistic vision to conduct experiments and concentrate on important matters. I am very lucky being one of his students. He has been a source of inspiration to learn and understand the subject more deeply, I am very thankful for his sincere support and constant encouragement. I am very grateful to **Mr Niraj Babu, Ms Ritika Chauhan, Ms Sruthi Unni** and **Mr Ravi Dadsena** for the informative discussions and creating a healthy workplace environment in lab. Finally I would like to thank all the staff of NITR for their support and valuable suggestions during my project work. I would also like to extend my deep gratitude to **Mr Patitapabana Parida**, Lab Technician for his help and ensuring that all the instruments are producing proper results and there was never a shortage of components and chemicals. This project work would not have seen this day of completion had there not been a constant support and encouragement from my family and friends **Karthic Krishnan, Saikat Kumar Sahoo** and **Neel Kamal**. I am deeply thankful to them.

M.Venkateswara Rao

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INTRODUCTION

1.1 Background

In the early years so as in 1994 in order to achieve Electroporation high voltage and short pulse was usually applied. But late Dr.Muramatsu, and Mr.Hayakawa, Nepagene, pursued to use mild conditions because high voltages usually damages the tissues. In 1998 however they tried to transfect adult chicken oviduct by applying pulses of 20-50ms with voltage 20-100V and were successful. Here they adopted the mechanism of low voltage and long square pulse. They were even successful in transferring the genes to chick embryos in vivo and presented a poster at the joint annual meeting of the Japanese biochemical society and the molecular biology society of japan in 1996, and published a paper in 1997. Electroporation system was applied to chick embryos for new culture in 2002 by Kobayashi. Electroporation can also apply to mouse embryos. It was first utilized to transfect mouse embryos in whole-mount culture in 2001 by Osumi and Inoue, and then to trace cell lineages in the mouse brain in 2001 by Tabata and Nakajima. Transfection of mouse embryos was achieved in an area-and time-specific manner and was applied to the study of telencephalon development in 2004 by Shinmogori [1]. Although relevant scientific observations were made since the 18th century, the electroporation phenomenon was not identified as an increase of membrane permeability until mid-20th century. After that, multiple applications of reversible electroporation emerged in vitro (DNA electro transfer) and in vivo (electro gene therapy and electro chemotherapy). Electroporation was tested commercially in the 1960s as a bactericidal method for liquids and foods but its use in the context of medical applications was not studied until the early 2000s as an ablative method. The first commercial system approved for clinical irreversible electroporation of soft tissues started to be produced in 2008 by Angiodynamics, Inc. under the brand name NanoKnife^{TM}. It consists of a high voltage pulse generator and single-use disposable electrodes.

1.2 Electroporator Mechanism:

Electroporation is a simple process in which host cells and selected molecules are suspended in a conductive solution, and an electrical circuit is closed around the mixture. An electrical pulse lasting a few microseconds to a millisecond, at an optimized voltage, is discharged through the cell suspension. This disturbs the phospholipid bilayer of the membrane and causes the formation of temporary pores. The electrical potential across the cell membrane simultaneously rises to allow charged molecules like DNA to be driven across the membrane through the pores in a manner similar to electrophoresis. Electroporator found a wide range of applications in present day life due to its compatible characteristic features. Electroporator is highly sensitive, costly device. It is usually used in molecular biology as a way of introducing small substances into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA. It is also used for the transformation of bacteria, yeast, and plant protoplasts [1]. This procedure is also highly efficient for the introduction of foreign genes in tissue culture cells (mammalian cells) as well as in tumor treatment, gene therapy, and cell-based therapy. The schematic diagram of Electroporator is shown in the Figure 1.1.

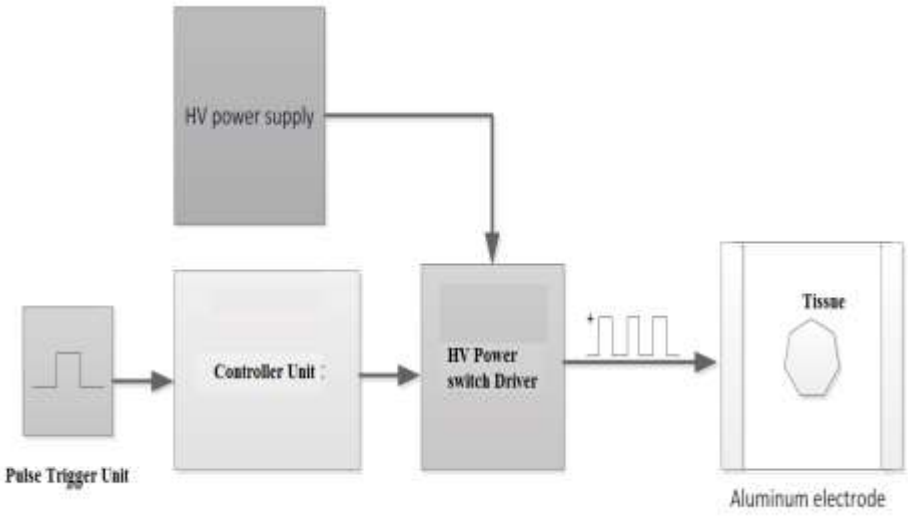

Figure 1.1: The pulse electric field system for electroporation [2]

1.3 How an Electroporator works?

In electroporation technique electrical current is introduced to a cell or group of cells. The electrical current disrupts the plasma membrane of the cells, creating openings where they did not exist before. If a substance is in solution with the cells, it will pass over the cell membrane after the electric current is introduced. Then, the cells can be allowed to rest and recover. In some cases, up to 85% of the cells may be successfully penetrated with the substance of interest. Once the cells get recovered, they may be cultured and examined to confirm that the electroporation was successful [34].

1.4 Existing Electroporators

1.4.1 Cryo Electroporator

Figure 1.2: Cryo Electroporator System [42]

Table 1.1: Cryo Electroporator System Description

Specification	Description
temperature	-5 degree~5 degree
input	AC110 V/60 Hz,AC220 V/50 Hz
power	150VA
Cooling temperature	-5 degree to 5 degree
electric current	8mA
size	39*30*29cm

1.4.2 Agile Pulse Electroporator (In Vivo System)

Figure 1.3: Agile Pulse Electroporator System [35]

Table 1.2: Agile Pulse Electroporator System Description

Specification	Description
User Interface	Touch Screen Display, Footswitch
Voltage Range	50 V to 1000 V
Pulse Width Range	0.050 to 10 ms
Pulse Interval	0.200 to 1000 ms (5 kHz to 1 Hz)
Data Export	USB Flash Key
Dimensions(with handle)	32 cm w x 20 cm h x 40 cm(12.6 in w x 7.9 in h x 15.7 in)
Weight	11.3 kg
Operating Temperature	10 to 40 deg.C
Mains Voltage	100 to 250 VAC
Fuse	5 Amp Slo-Blo®, 5 mm x 20 mm

1.4.3 Eppendorf Electroporator System (for transformation of bacteria & yeasts)

Figure 1.4: Eppendorf Electroporator System [36]

Table 1.3: Eppendorf Electroporator System Description

Specification	Description
Input voltage	115 V/50 Hz–60 Hz
Input current:	<0.5 A
Capacitor:	10 μF, 2,500 V pulse discharge
Time constant:	5 ms nominal with a sample impedance of 3.3 kΩ
Pulse voltage:	200 V–2,500 V
Charging time:	<8 s
Interface:	RS-232
Dimensions (L x W x H):	10.75 x 8.8 x 4 in/27.3 x 22.4 x 10.16 cm
Weight:	2.74 kg

1.4.4 Gene Electroporator (Scientz-2c)

Figure 1.5: Gene Electroporator System [37]

Table 1.4: Gene Electroporator System Description

Specification	Description
Model:	Scientz-2C
High Output Voltage:	400-2500 V
Low Output Voltage	100-450 V
High Voltage Capacitor	1, 5, 6, 25, 30, 31uF
Low Voltage Capacitor	100 CF, 125 µF, 150µF, 1675µF one grades of 25µf
Resistance	50,100,150,1600; total30 grades
Operating System	Microcomputer control
Output waveform	With RC time constant of the exponential decay of wave
Dimension	36.8 X 31.6 X 22.9(cm)
Net Weight	10.5 Kg
Packing size	480X420X280mm

1.4.5 Micropulser Electroporator System

Figure 1.6: Micropulser Electroporator System [38]

Table 1.5: Micropulser Electroporator System Description

Specification	Description
Input voltage	In-line switching, 100–120 V or 220–240 V
Input current	2 amp RMS (100–120 V) 1 amp RMS (220–240 V)
Maximum output voltage and current	3,000 V peak into > 600 ohm Limited to 100 A peak maximum
Output waveform	Truncated exponential waveform with RC time constant of 5.0 ms assuming loads of 3.3 KΩ
Output voltage adjustment	200–3,000 V range with 10 V adjustment
Pulse-time adjustment	In manual mode, set time of range 1.0–4.0 ms with 0.1 ms precision (provided that the sample-determined pulse width has time constant >4.0 ms)
Operating environment	Temperature 0–35°C, Humidity 0–95% without condensation
Dimensions	31 x 21 x 8 cm (L x W x H)
Weight	2.9 kg

1.4.6 Invitrogen Electroporator (NEON Electroporator)

Figure 1.7: Invitrogen Electroporator System [39]

Table 1.6: Invitrogen Electroporator System Description

Specification	Description
Input Power	100–240 VAC, 3.0-6.0 Amps, 50–60 Hz, 300 W
Output	0.5-2.5 kV
Pulse Width	1-100 ms
Maximum Duty Cycle	0.1
Charging Time	Maximum 8 seconds
Altitude	Up to 2,000 meters
Operating Temperature	5ºC to 40°C
Maximum Relative Humidity	Up to 80%
Degree of Protection	IPX0
Protective Earthing	Class I (earthed)
Installation Category	II
Instrument Type	Bench top unit
Device Dimensions	9.2 inches (w) × 11.8 inches (l) × 8.66 inches (h)
Pipette Station Dimensions	5.91 inches (diameter); 5.51 inches (h)
Device Weight	6 kg
Built-in Features:	Touch screen (800 × 480 pixels), digital display

1.4.7 ECM 830 Electroporator System (For In vivo Applications)

Figure 1.8: ECM 830 Electroporator System [40]

Table 1.7: ECM 830 Electroporator System Description

Specification	Description
Operational Status:	Internal self-test upon start-up
Interface:	Digital User Interface
Input:	110 V/220 V Universal
Charge Time	5 sec maximum (without delay)
Pulse Length	Range 10 μs – 999 μs LV Mode/ 1 μs resolution1 msec – 999 msec LV Mode/ 1 msec Resolution 1 sec – 10 sec LV Mode/0.1 Sec resolution 10 μs – 600 μs HV Mode/ 1 μs résolution
Voltage Range	5 – 500 V LV Mode/ 1 V resolution 505 – 3000 V HV Mode/ 5 V resolution
Multiple Pulsing	1 – 99
Pulse Interval	100 msec – 10 sec
Programmability	Storage for 3 protocol setups (V, t, n, interval)
Arc Control	Arc Quenching
Safety	Generator short circuit proof
Capacitance	4000 μF LV, 111 μF HV
Input	500 A limit at 100 μs
Remote Operation	Footswitch available

1.4.8 Cuy21edit- Electroporator (In Vivo)

Figure 1.9: Cuy21 edit- Electroporator System [41]

Table 1.8: Cuy21edit- Electroporator System Description

Specification	Description
DC Wave	Square
Voltage	1～500 V
Pulse time	0.1～999.9ms
Pulse Interval	0.1～999.9ms
Pulse number	1～99 time(s)
Impedance measurement	~18kΩ
Effective voltage	1～500V
Effective current	0.01～4.00A
Memory track	2 tracks
Power	Single phase 100V,5A,50/60Hz
Size	W390cm×L450cm×H250cm

1.5 Objectives

The objectives of this project comprises of accomplishment of the following parameters

1. To design and fabricate a low cost glass slide based electroporator system with small foot print.
2. To equip the system with a high precision high voltage generator so that the applied voltage can be varied between 300 to 1000 V (it will enable the system to transform wide variety of cell lineages)
3. To integrate the system with a high precision pulse generator to obtain a range of 1 to 1000 pulses per second (or pulses with duration from 10µs to 1000ms).

CHAPTER- 2
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LITERATURE REVIEW

2.1 Modelling and Simulation of Electroporation System with Measured Bioimpedance

Warindi et al reported that electroporation is a set of techniques of giving small materials into biological cells by use of a high pulsed electric field. The success of electroporation is determined by parameters, procedure and the tissue properties. The cell is modelled as a medium with conductivity and permittivity. Both these are measured by an LCR meter. The media is modelled as a conductive film to get a mathematical model in the form of partial different equation problems. A Finite Element Method (FEM) is used as a way to solve the issue. A recent use of pulsed electric field in medicine is electroporation. Electroporation occurs when electrical energy is induced into cells to increase the permeability of cell membrane which leads to formation of membrane pores. Those pores are then used to make organic molecule, gen, antibody peptide, or DNA to enter into cells. Electroporation can also be used for combining different cells. In reversible electroporation (RE), cell membrane pores usually immediately close when electric field disappeares [4]. If electrical energy in the pulse is too large or too long, the cell will be damaged due to injured cell membrane. It is called irreversible electroporation (IRE). The schematic mechanism of electroporation is shown in Figure 2.1

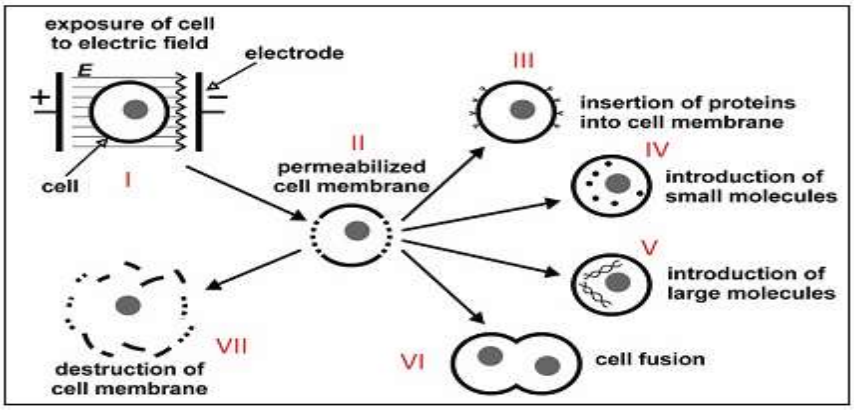

Figure 2.1: Cell Electroporation Mechanism [4]

In vivo electroporation is used for research because it is the most valuable but a risky procedure. Until now the existing electroporation method was based on practical experience from experimentations. The problem was to know that electroporation success was not attained as needed, considering the minimization of side effects; therefore it was necessary to conduct research for electroporation through numerical calculations to determine the ultimate parameters of electroporation. The research gives a simulation of pulsed electric field distribution at tissue in order to know the part of tissue that the cells are electroporated. It will also determine the electroporation parameters that give the details of electroporated area. The results are expected as design guide of safe but successful electroporation system.

2.2 Characterization of Electroporator Systems

2.2.1 Polarity

Janja Dermol et al have found that a membrane's selective permeability is affected by the electric pulses given at high voltage. The primary reason for this, according to theory is that pores are formed in the membrane and thus they become permeable to different kinds of molecules which in many other cases not be possible. It is observed that if this transmembrane voltage exceeds a certain threshold, there is an increase in the membrane permeability. The membrane permeability is also found to depend on pulse parameters. There are basically two kinds of electroporation, one is known as reversible electroporation and the other irreversible electroporation. The process is termed as reversible electroporation if the cell can recover after it has been exposed to electric pulses whereas if the cell is not able to recover and is not able to survive the electroporation it is termed as irreversible electroporation. Electroporation has been used in a wide variety of fields such as cancer treatment, biotechnology, gene transfer and food processing [5].

From this knowledge about electroporation it can be said that the cells are assumed to be electroporated if the transdermal voltage induced exceeds the characteristic threshold voltage and if the transdermal voltage is below a certain threshold voltage the cells are not electroporated. Transitions from normal state to reversibly and irreversibly electroporated state is always continuous. By the use of the specific electrode positioning a certain amount of voltage can be applied and by selecting a suitable mathematical model the percentage of cells are being effected by the voltage can be predicted.

It is essential to define the duration of the pulses and the voltage of the pulses that is being applied. For this the geometry of the electrodes and the cells are modelled as a 2D tissue layer. Electric field (E) distribution is calculated numerically in order to determine the adequate voltage that has to be applied. As the tissues and electrodes are complicated to understand in most of the cases analytical calculations are not done. There is no established conventional or an easy way to measure electric field in vivo[6].

2.2.2 Electric Field

In living cells however there are some techniques which are predicted based on current density imaging (CDI) and magnetic resonance electrical impedance tomography (MREIT) to measure electric field. However numerical modelling method is considered to be the more reliable and an easy way [7].

The point of our study was to foresee a rate of electroporated cells developed as a thick monolayer laid open to an inhomogeneous field. We performed examinations in a homogeneous electric field and decided the rate of cell electroporation for a certain connected E. We utilized these results within four diverse numerical models, which introduced and extrapolated the rate of electroporated cells to different estimations of E. We utilized homogeneous E for computing parameters of the numerical models in light of the fact that it is conceivable to focus the Percentage of rate of electroporation. We utilized the

inhomogeneous electric field for acceptance on the grounds that in tumors and tissues the electric field around the anodes is in very nearly all cases electroporated cells at a certain E. We accepted the numerical models by laying open cells to the inhomogeneous field and analyzing anticipated and tentatively decided qualities inhomogeneous. The anticipated qualities were acquired by utilizing the numerically figured inhomogeneous E in numerical models. We got the rate of electroporation in the reliance on E for the territory around the cathodes.

The focal point of this methodology is that straightforward cathode geometry setups might be utilized to figure the parameters of the numerical models. Numerical models foreseeing cell electroporation can then be connected to discretionary terminal geometry. This sort of scientific relationship could permit us to present medication arranges in a clearer and more justifiable way. Right now, the medication arrangements display the E field connected to a certain territory of a tissue/tumor. Utilizing this technique the rate of the electroporated range of a tissue/tumor could be indicated. In the long run additionally the number and span of beats could be considered.

2.3 Electroporation of Cell Membrane

T.kotnik et al expressed that each one body has about trillions of organic cells which is thus secured by a plasma film. The primary trademark capacity of a cell plasma film is to discrete and secure the cell from its nature's domain. It is basically made out of a bilayer of lipids which are something like two particles in thickness. It has conduct amidst that of a gel and a fluid. Within a lipid bilayer is likewise steady and typically holds an extensive number of distinctive sorts of proteins, few of which additionally go about as diverts and pumps helping in the transport of the atoms over the films. Without the vicinity of the proteins the layer might be considered as an expansive hindrance which is practically impervious. Nonetheless, electrically the phone plasma film could be considered as a slight protecting sheet

encompassed by watery electrolyte results on both sides. The electric breakdown might be seen in the film subsequently prompting the transportation of the particles crosswise over it which is generally impervious if a sufficiently solid electric field is connected to the layer. This procedure is generally termed as film electroporation [8].

On the in spite of the insulator encasings where an electric breakdown typically causes changeless changes in the structure, on account of a layer where the lipids act as a 2d fluid, can come back to its pre breakdown state spontaneously. Electroporation has been widely utilized via specialists as a part of numerous fields, for example, solution and biotechnology. Today, reversible electroporation is a created system for bringing chemotherapeutic pills into tumor cells (electro-chemotherapy). It additionally offers incredible guarantee as a system for gene help without the dangers brought about by popular vectors (DNA electro exchange). In clinical medication, irreversible electroporation is continuously researched as a strategy for tissue removal (non-warm electro removal), inasmuch as in bio-innovation, it is helpful for extraction of biomolecules and for microbial deactivation in food conservation [9].

Electroporation clarifies the system at atomic level of the lipid bilayer, and afterward proceeds to the cell level.it will clarify how introduction of a cell all in all to an outside electric field brings about an actuation of voltage on its plasma film, and transport intensive the electroporated layer.

2.4 Electroporation optimal Parameters for Gene and tissue

Electroporation is a non-viral or physical gene delivery approach which utilizes electric field to create pores on the cells (microbial, plant or animal cells). Creation of pores leads to the passage of DNA across the cell membrane and reach the nucleus. Ideal parameters for the successful transportation of DNA include high voltage for short pulse duration. To achieve

this, various scientists tried different approaches *viz.*, electroporation gadgets by Eberhard. N. In general, electroporation do not damage cell structure and is a non-invasive technique [2].

2.4.1 Gene Delivery by Electroporation

Transferring genes into tissue has been performed by electroporation. The research has been centered fundamentally on the phenomenon of reversible and irreversible layer breakdown of cell membrane *in vivo*. The electroporation technique facilitates in conveyance of remote materials (gene, DNA, and RNA) into single cell and tissue [10]. Electroporation incorporates high quality electric field with short pulses. These advantages provide an alternative to the standard gene transfer systems *viz.,* retro infections and adenoviruses. On the other hand, electroporation is a non-popular technique. Electroporation helps in transferring the genes to tissue *in vivo* or cells *in vitro*. Till date, electroporation was employed to transfer genes in creatures, for example, rodent liver, rodent cerebrum tumors and mouse skin [11].

2.4.2 Physical Mechanism of Electroporation

Fig. shows the parameters of electric field and pulse width of electroporation. In general, applied electric field ranges between 10^3 and 10^4 V/cm, and pulse width lies around 10^{-3} to 10^{-4} second for the passage of genes [12]. For pill passage, electric field is kept at about 10^3 to 10^4 V/cm and pulse width is associated for 10^{-4} to 10^{-5}sec. This indicates that electric field and pulse width are being the key parameters for the electroporation technique. A perfect of electroporation technique involve the principles of DNA transfer and atomic science without any symptoms. Since electric energy has been employed there is a potential chance for skin burns, infections and immunological impacts [13].

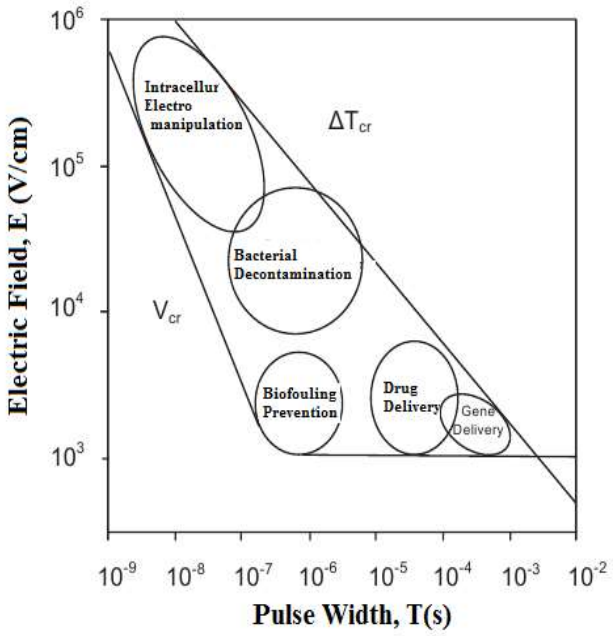

Figure 2.2: Parameters of electric field and pulse width[2]

2.4.3 Process of Electroporation with Genes:

This section deals with the methodology of electroporation for gene transfer (Fig 2). For gene transfer, electroporation involves four steps. First step is to apply an electric field to cells or tissues, followed by the application of electric field into cells. At this stage, pores will be created and the genes will be transferred. Third step involves, electric pores structuring when the right parameters of electric field force and pulse width are maintained. For eg, the most widely recognized electric field for transfer of genes in to human blood cells is between 1 and 2.4 kV/cm. At this condition, high cell reasonability and transfection proficiency can be obtained. To achieve better results, pulse width was maintained for 100 μsec. After application of electric field, pores will be opened for a couple of or one microsecond depending on the exposure time. Finally, with the removal of electric field, the pores will reseal and completes the electroporation [14].

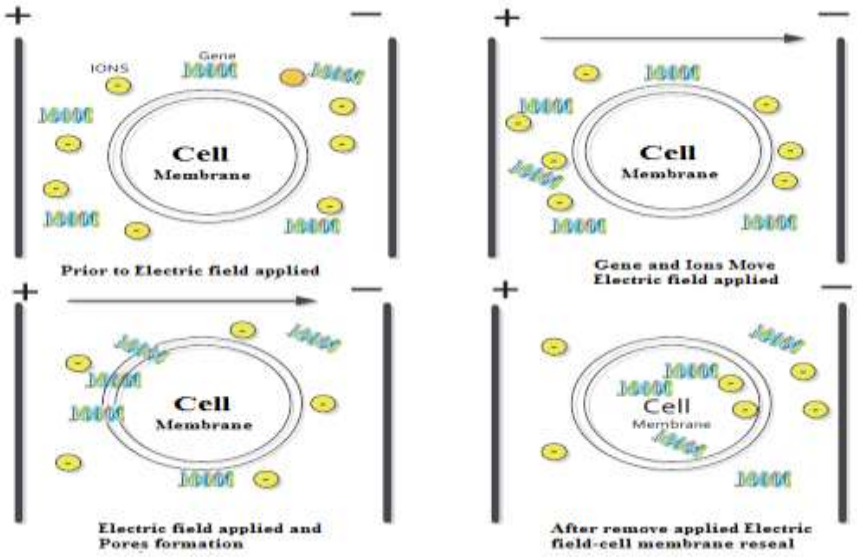

Figure 2.3: The process of electroporation[2]

2.4.4 Optimal Electric Field Parameters:

The relationship between the ideal parameters (electric field and pulse time) was indicated in Fig. 3. The un-electroporated stage involves that the electric field beat abundance is less than the base voltage for cell film break down (0.7volt). This voltage is not enough to open the electric pores and penetrability of genes in to cells. At the next stage, electroporation involves high pulse voltage for cell membrane break down so that the genes could pass through the pores in to cells. The optimized conditions for gene transfer involves 50 V/cm for 20 msec. In this regard, medication will be given at 1 kv/cm for 100µsec. During cell lysis, the electric field expands, but penetrability of cells may not happen. Under these conditions, some of the cells may undergo lysis. To prevent cell lysis, the parameters need to be employed near the area of sort of gene conveyance and medication conveyance

[15].

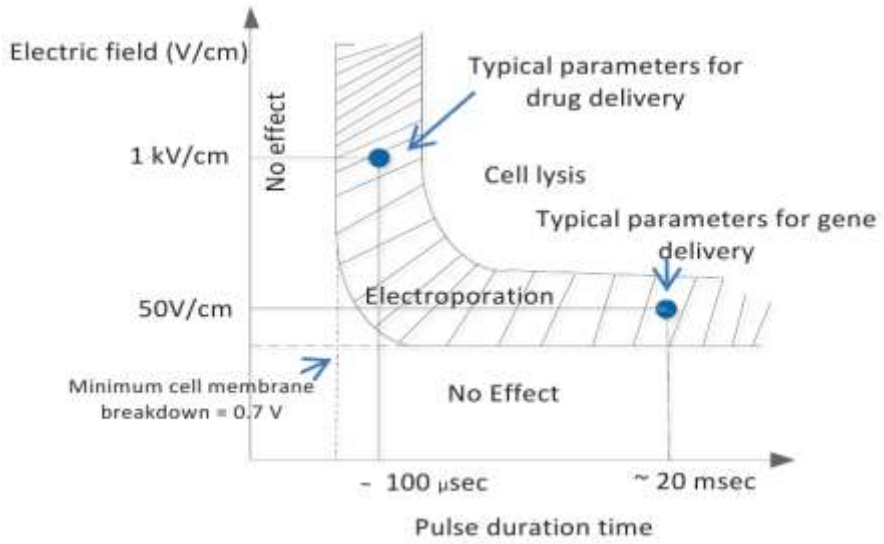

Figure 2.4: The relationship parameters among electric field and pulse duration time[2]

2.4.5 Pulse Electric Field System

The key to the success of electroporation is the framework of pulsed electric field. Hence, the fundamental field framework needs to be understood. It consists of a pulse trigger unit, a controller unit, a high voltage power supply, a high power switch driver and a chamber (aluminum terminal) as demonstrated in Fig. This set up creates the electric field and pulse for a span of time. The pulse width ranges from 100 μsec to 100ms depending upon the gene and medication conveyance. The controller unit helps in controlling the amount of beat and length of time. To achieve this, high power supply is utilized ranging between 100 Volt and 1000 Volt. High power supply is associated with high power switch, and the capacity of high voltage switch ranges between 100 Volt and 1000 Volt. This is connected to the test chamber which consists of parallel aluminum terminals with a dividing crevice from 1 cm. to 4 cm. Furthermore, tissues could be placed in the test chamber. By employing these conditions, gene transfer need to be done without cell lysis and cell harm [16].

2.4.6 Applications of Electroporation In Gene And Tissue

This area examines the requisitions of electroporation in gene and tissue. It might be seen that this came about because of diverse sorts of tissues, for example, chick and mouse. Single cell

line can likewise be fruitful in-up taking gene into Hela cells under electroporation conditions. It could be perceived that transfection in both tissue sweep be accomplished focused around the low electric field quality extending from 10 to 90 Volt. Then again, the gene transfection in both sorts of single cells could be accomplished; furthermore, the electric field is ordinarily connected as a solitary or different short (ms) beats to tissue. It might be watched that the transfection of Hela cells after connected electroporation at electric field quality of 600 V/cm and beats length of 10 ms as demonstrated in Fig.6. Additionally, electroporation of tissue has been exhibited for focusing on conveyance of chemotherapeutics to tumors, productivity gene transfection of cells in vivo, and expanding skin penetrability for transdermal medication conveyance [12].

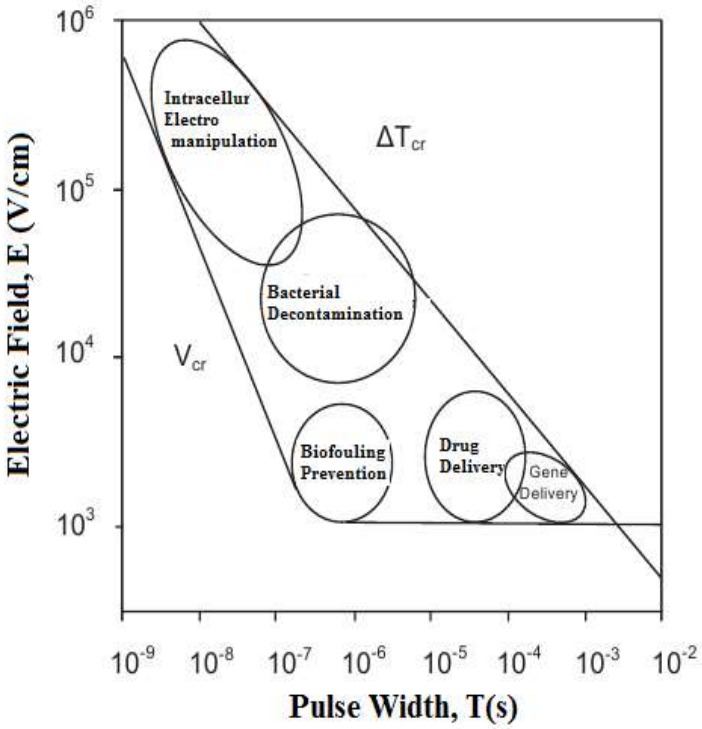

Figure 2.2: Parameters of electric field and pulse width [2]

2.5 Equivalent Pulse Parameters for Electroporation

Gorazd Pucihar et al uncovered that Electroporation-based requisitions oblige the utilization of particular beat parameters for an effective conclusion. At the point when proposed estimations of beat parameters can't be set, comparable results might be gotten by utilizing identical beat parameters. We decided the relations between the sufficiency and term/number of beats bringing about the same portion of electroporated cells. Beat span was changed from 150 ns to 100 ms, and the amount of beats from 1 to 128.with longer beats or higher number of beats, more level amplitudes are required for the same part of electroporated cells. The statement determined from the model of electroporation could depict the measured information all in all interim of beat spans. In a narrower run (0.1–100 ms), less perplexing, logarithmic or force capacities could be utilized. The connection between plentifulness and number of beats could best be portrayed with a force capacity or an exponential capacity. We demonstrate that generally straightforward two-parameter force or logarithmic capacities are valuable when proportionate beat parameters for electroporation are looked for. Such scientific relations between beat parameters could be paramount in arranging of electroporation based medicines, for example, electro chemotherapy and non-warm irreversible electroporation [17].

The living tissue is demonstrated as a medium with conductivity and permittivity. Both properties are acquired from estimation of the impedance. The entire electroporation framework structures an electromagnetic framework which showed up as scientific model of halfway differential comparison issues. A limited component technique (FEM) is utilized as a device to take care of and mimic the issue. FEM gives graphical presentations indicating the potential electric field appropriation. A guide of electroporation that focused around electric field presentation is then examined to acquire an electroporation range. The results are

electroporation parameters in term of cathodes potential diverse and separation, length of time, number, and interim of the beats. It is possible by picking the particular parameters that transform certain electroporation region. A few sets of parameters that generate an extensive variety of electroporation range are likewise exhibited.

2.6 High Efficiency Electro transfection

U. Friedrich et al reported that Electro permeabilization or electroporation has been utilized generally for joining of xeno particles (up to the span of DNA) into eukaryotic cells without misfortune of cell capacities. Often, ease gear is utilized which conveys exponentially rotting field beats of low force and millisecond term time. This together with the utilization of disposable cuvettes furnished with aluminum plate anodes can extensively diminish the survival of the cells and the fuse of xenomolecules on account of the unfavorable symptoms of long-span beats and the lethality of Al3q-particles on permeabilized cells. Nuclear assimilation spectroscopy demonstrated that generous measures of Al3q-particles (up to 1 mm) were solubilized from the cathodes after beating. Solubilization of Al3q-particles happened due to the progressions of the ph near the terminals actuated by electrolysis of water. The neighbourhood ph-progressions could be imagined by the expansion of ph-markers together with agarose to the beat medium. Interestingly, when utilizing short beats of 40 ms to 100 ms length of time, cell lines indicated that generally high survival rates of cells and species-particular transfection yields (of up to 50–70% as measured by FACS analysis)could be gotten by utilizing aluminum anodes gave that brief time beats were connected. These yields were in the extent got by utilizing stainless steel cathodes. The obliged brief time beats and field qualities for field-prompted fuse were attained utilizing a novel power supply (greatest yield voltage of 1.2 kv, beat spans between 15 ms and 500 ms) in synthesis with unequivocally hypo-osmolar beat media [18].

CHAPTER- 3

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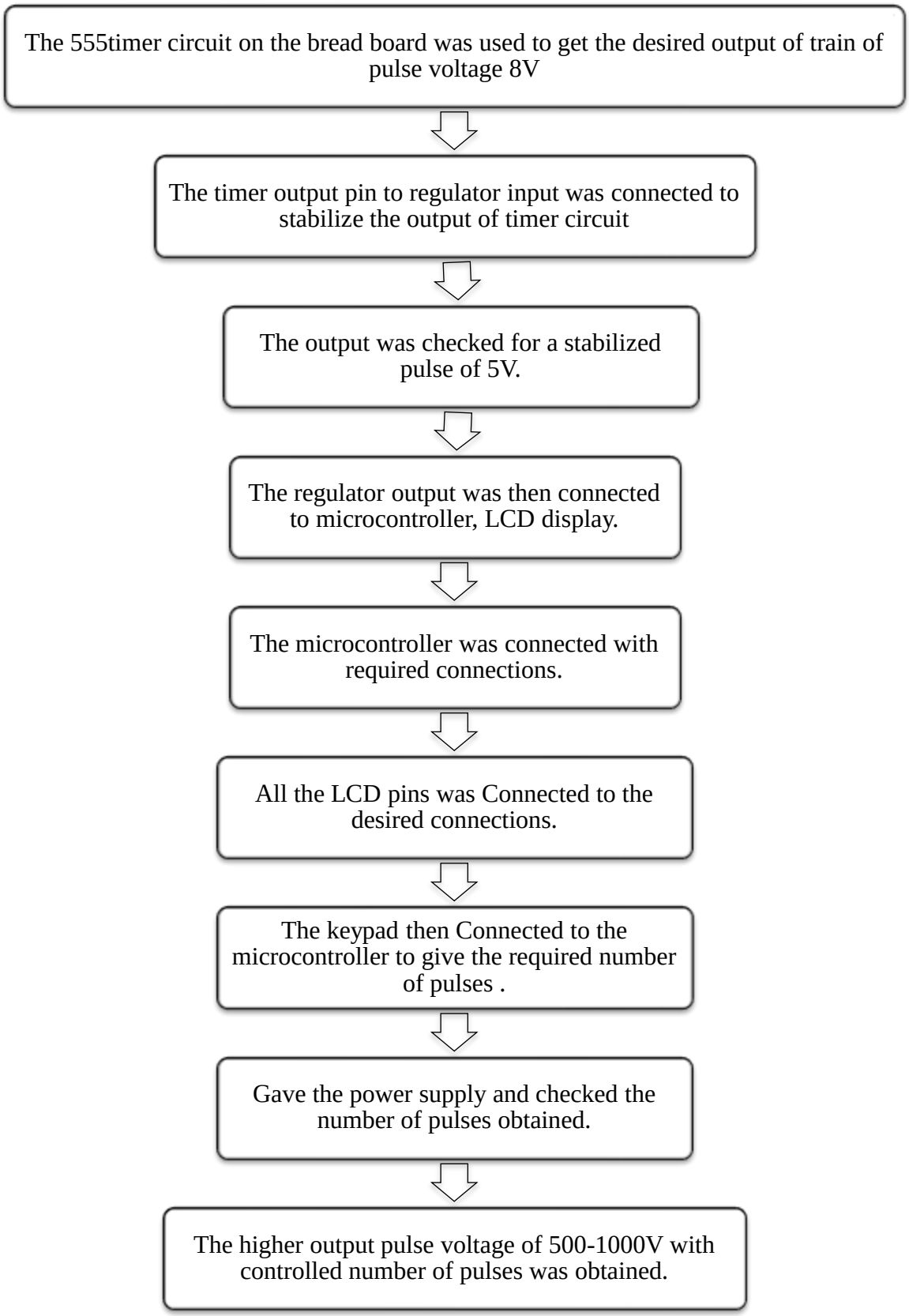
MATERIALS AND METHODS

3.1 WORK PLAN:


The 555timer circuit on the bread board was used to get the desired output of train of pulse voltage 8V
The timer output pin to regulator input was connected to stabilize the output of timer circuit
The output was checked for a stabilized pulse of 5V.
The regulator output was then connected to microcontroller, LCD display.
The microcontroller was connected with required connections.
All the LCD pins was Connected to the desired connections.
The keypad then Connected to the microcontroller to give the required number of pulses .
Gave the power supply and checked the number of pulses obtained.
The higher output pulse voltage of 500-1000V with controlled number of pulses was obtained.

3.2 MATERIALS AND METHODS

Components

1) Bread Board
2) 7805 Regulator
3) 555 Timer
4) Transformer
5) LCD Display
6) Battery (9V)
7) Crystal (12 MHz)
8) Relay(12V)
9) Resistors (1K, 10K, 100K)
10) Potentiometers (10K)
11) Micro Controller-89C52
12) Capacitors (10µF, 33pF)
13) Connecting Wires
14) Electrodes
15) Customized Glass Slide

Instruments

1) Digital Storage Oscilloscope

2) Microcontroller Programming Kit

3.2 FABRICATION AND DESIGN OF MATERIALS

3.2.1 Bread Board

A bread board was used for assembling the circuits on an insulating surface, mainly with solder less contacts, where components can be easily changed with circuit alteration and experimentation. In other words this was a constructional base which was meant for a unique kind of electronic circuit known as a prototype. It did not require soldering hence was reusable. This enables creating temporary circuits for the purpose of experiments [19].

Figure 3.1: Design of Breadboard Device

3.2.2 7805 Regulator

The LM7805 regulator was used to step down the DC pulsating voltage from voltages of higher value to less than 5V DC. The best part about the 7805 was that it was very common and very cheap.

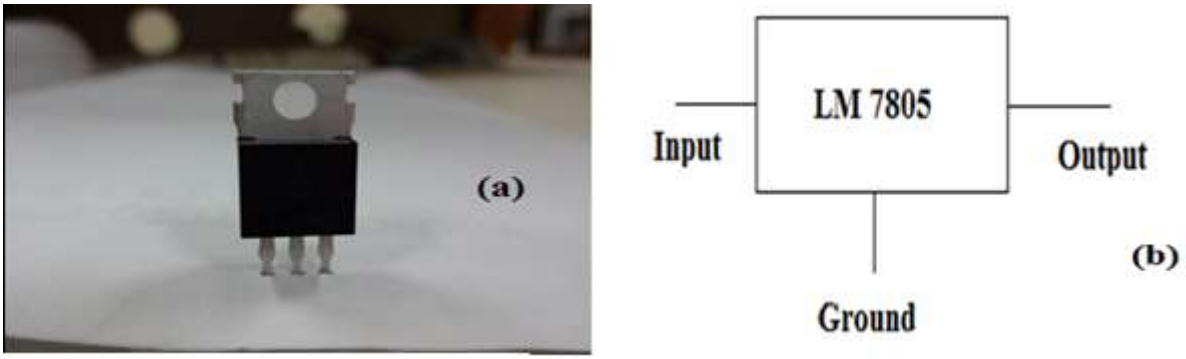


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Figure 3.2: (a) Regulator Component (B) Design of Regulator Device

3.2.3 555 timer circuit

The 555 timer IC was used for micro pulse generation and regulation of pulse duration for the Electroporator. It was used to obtain the continuous pulse waveform of 9V.

Figure 3.3: 555 Timer Device

The circuit that was used in creating the pulse waveform of 9V with the help of 555 timer circuit is shown in Figure 3.4 [20].

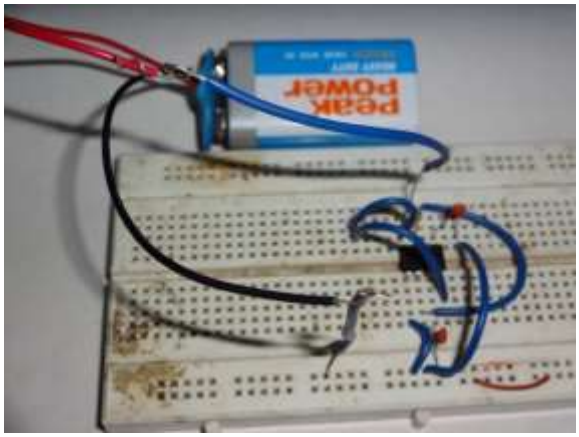

Figure 3.4: 555 Timer circuit

3.2.4 Transformer Circuit

In the current project, the goal was to provide a high alternate current pulse (few hundreds of Volt) to live cells for creating temporary pores on cell surface so that various biomolecules can be introduced in to the cells. Thus, a transformer (Metronix Pvt Ltd., India) was essential to get a high pulsating output voltage from a low pulse input voltage. To obtain this high output pulse voltage of 500-1000V transformer of turn ratio 80-100 was used. A transformer

was utilized as a secure and effective voltage converter to alter the AC voltage at its input to a lower or higher voltage at its output without altering its frequency [21].

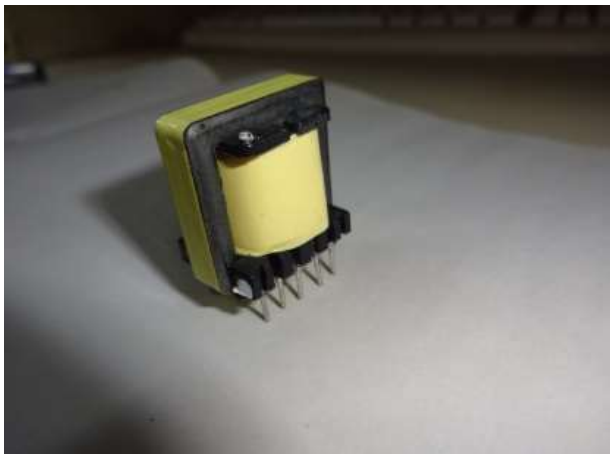

Figure 3.5: transformer device

3.2.5 LCD Display

Liquid-crystal Display (Formike Electronic Co., Ltd, India) was used as a video display to show the number of pulses given through keypad. When the battery was given power then LCD was in on-mode and when it was given the required number of pulses through keypad it showed it on display directly [22].

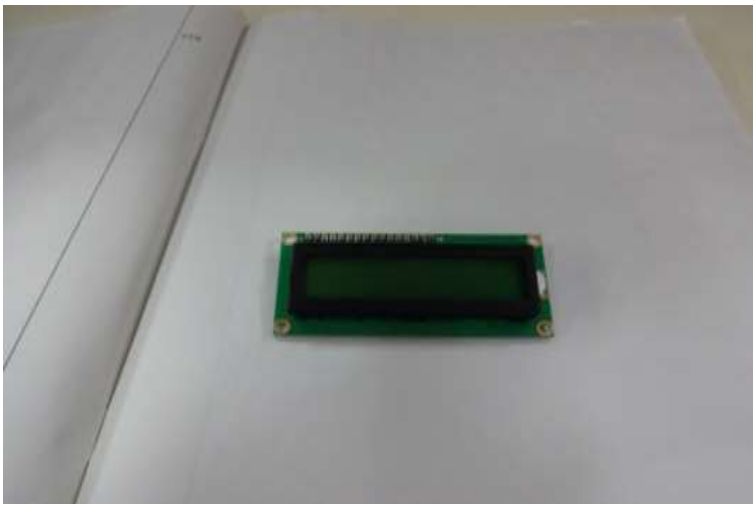

Figure 3.6: Liquid Crystal display device

3.2.6 Lithium-ion battery

A Li-ion battery of 9V (Peak Power Services, Inc., India) was used as power source to the 555 Timer and LCD circuits. The battery can supply a constant DC voltage of 9V to the designed circuits to become operational [23].

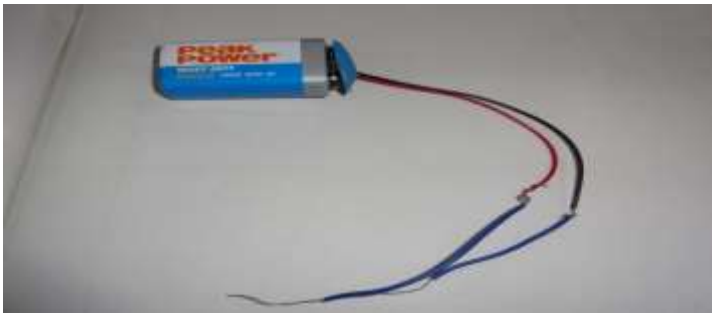

Figure 3.7: Battery component

3.2.7 Crystal 12 MHz

A quartz crystal (SPK Electronics Co., Ltd., India) was used with an electronic oscillator circuit due to its piezoelectric property. It can continuously monitor the fluctuations in frequency and supply an electrical signal with a very precise frequency of 12 MHz that can match with the Microcontroller input. This specified frequency has commonly been used to provide a stable clock signal for digital integrated circuits, to stabilize frequencies for micro-controllers and to keep track of time [24], [25], [26].

Figure 3.8: Crystal (12 MHz) Device

3.2.8 Capacitors

Appropriate capacitors (RAM Electronics Ltd., India) were used to create the required time period of the pulsating DC output. These components also assisted in tuning the micro-controller circuit with help of Quartz crystal. Capacitors of 10uF, 33pF were used for continuous pulse generation. It was a passive two-terminal electrical device was expressed as the ratio of the electric charge (Q) on each conductor to the potential difference (V) between them. Farad (F) is the SI unit of capacitance [27].

Figure 3.9: Capacitor Components

3.2.9 Resistors

Resistors (Cermet Resistronics Pvt. Ltd, India) of 10KΩ, 100KΩ, 1KΩ were used to create the time constant for the pulse wave form in determining its frequency. It was also used in powering the LCD with battery to avoid damage [28].

Figure 3.10: Resistor Components

3.2.10 Potentiometers

A potentiometer (Bochen Chengdu guosheng technology co., Ltd., China) of 10KΩ was used to change the output wave form frequency from 50 µs to 1000 ms. It was also used in changing output waveform peak to peak voltage from 10 to 800V. In general, it was a resistor with three terminals for providing the required frequency and output voltage range by triggering its second terminal [29].

Figure 3.11: Potentiometer Component

3.2.11 Microcontroller-89C52

Microcontroller (ATMEL Integrated Circuits, America) was used to control the number of pulses given by the 555 timer circuit output. 7805 regulator was used to step down the output of pulsating voltage from 9V to 5V in order to bring in the Microcontroller range. Now, its output was given to a 12V relay for controlling the number of pulses required. Microcontroller helped in switching the relay on/off depending on the number of pulses given through keypad. An ideal Microcontroller includes a processor, memory and peripherals. Typical applications of a Microcontroller include regulating the operation of highly sensitive devices where a precise output value is required. A compact Microcontroller 89C52 was designed to govern the operation of embedded systems in Electroporator [30].

Figure 3.12: Microcontroller Device

3.2.12 Connecting Wires

Connecting wires are electro-mechanical devices to join electrical circuits as an interface. The connections might be temporary for bread board but used in connecting the different electrical components in our Electroporator design circuit.

3.2.13 Relay

Briefly, a relay of 12V (Zhejiang Zhongji Technology Co., Ltd, China) was used for controlling the number of pulses of the electroporator system and also to regulate pulse waveform. When the relay was on, a continuous train of pulses were generated. When the relay was off, pulse generation was stopped. The number of pulses and pulse waveform as per the user's requirement was controlled by the microcontroller. The microcontroller's input was given from regulator output while the output was displayed by a digital storage oscilloscope [31].

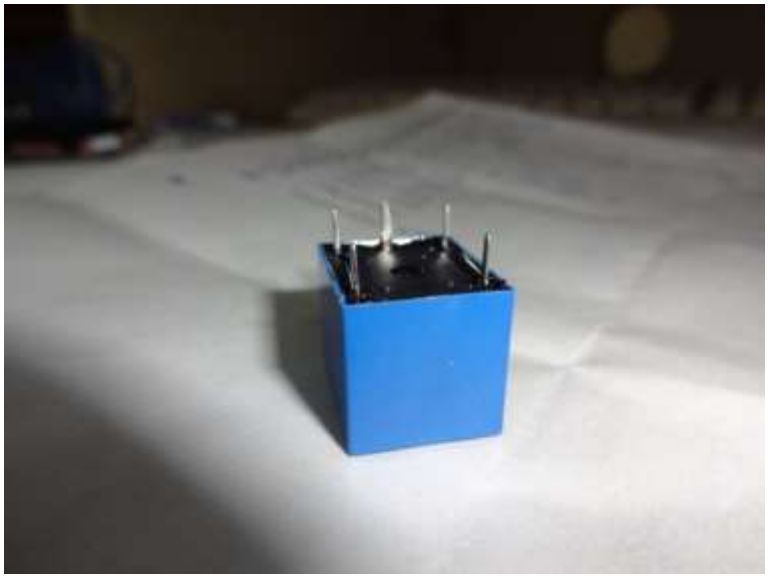

Figure 3.13: Relay (12V) Device

3.2.14 Customized Glass Slide

The housing of cell suspension was prepared from a thick glass slide (100 mm x 100 mm x 20 mm: L x B x H). Briefly, a circular well of 25 mm diameter and 14 mm depth was extruded from the centre of the glass slide with the help of round glass cutter. The circular well was used for keeping the cell suspension to be transfected. The well can accommodate a solution of 5-7 ml.

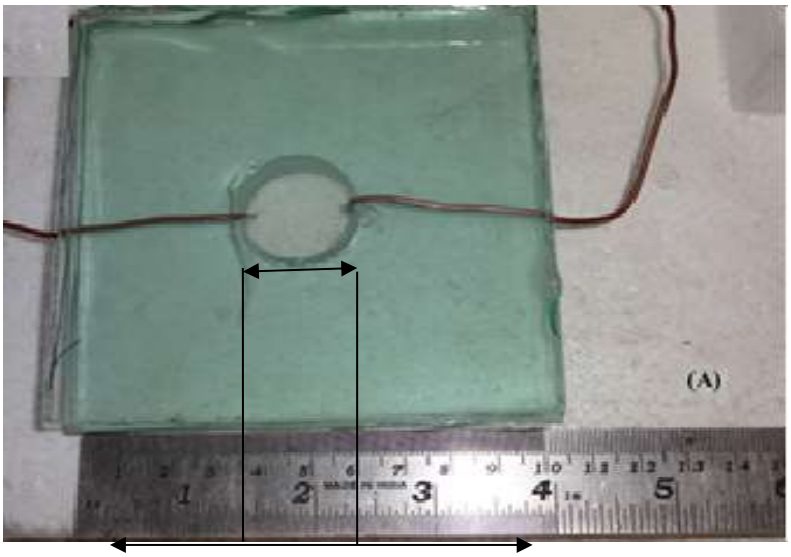

Figure 3.14: Customized Glass Slide top-view

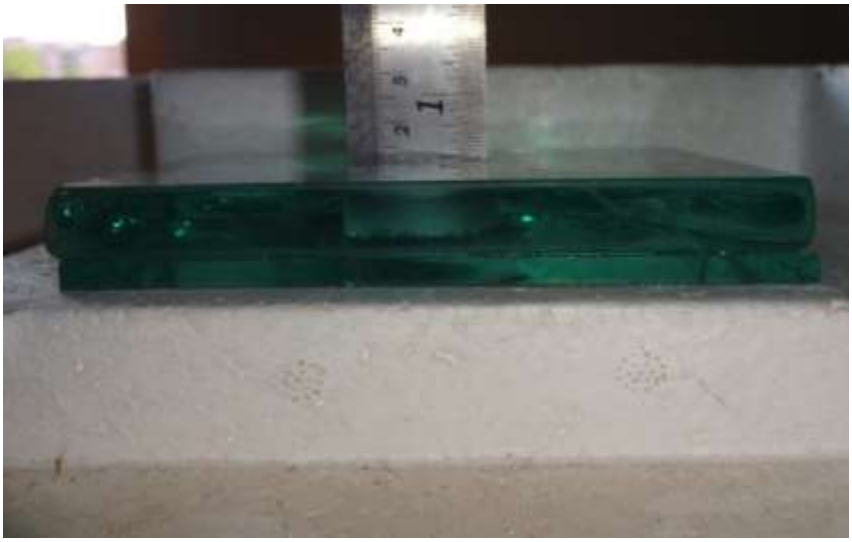

Figure 3.15: Customized Glass Slide Side-view

3.2.15 Electrodes

An electrode was an electrical conducting wire used to make contact with a non-metallic part of a circuit [32].

Figure 3.16: Metallic Electrode

3.3 INSTRUMENTS

3.3.1 Digital Storage Oscilloscope

A digital storage oscilloscope (Tektronix *India Private* Limited, America) was used to analyze and store the desired outputs from 555 timer circuit, 7805 regulator, 12V relay circuit, micro-controller circuit and transformer circuits. It was the most usual type of oscilloscope in market because of the storage, advanced trigger, measurement features and display which it typically provides. Its operation started with the input analogue signal sampled and then converted it into a digital record of the amplitude of the

signal at each sample time. It provided the sampling frequency not less than the Nyquist rate to avoid aliasing [33].

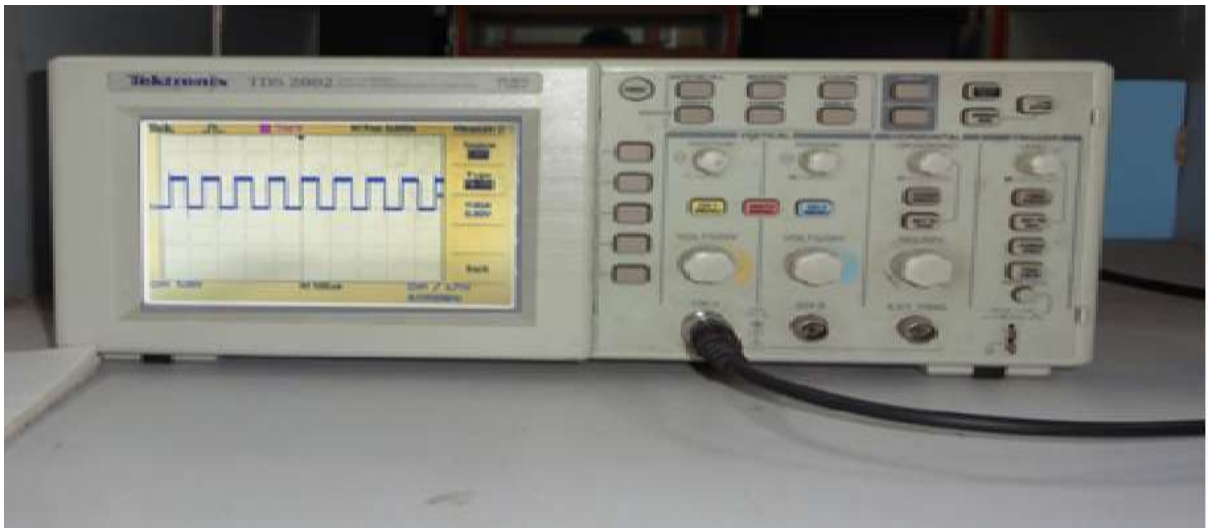

Figure 3.17: Digital storage oscilloscope instrument

3.3.2 Microcontroller Programming Kit

This programme kit (Pantech Prolabs India Pvt., Ltd.) was used for feeding the programme into the Microcontroller (89C52). The programme was written in C++ language on keil (µ vision4, UN, USA) and its HEX file was created to make it legible for reading by the Microcontroller (89C52).

Figure 3.18: Microcontroller Programming kit

CHAPTER- 4

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RESULTS AND DISCUSSION

4.1 SIMULATION RESULTS

555 IC chip was placed on the bread board. The 8th pin was connected to source and 1st pin was connected to ground. Next, 2nd, 6th pins and 4th, 8th pins were shorted. Two capacitors of 0.01µF each were connected between 2nd pin to ground & 5th pin to ground. A resistor of 10KΩ was connected between 4th, 7th pins and another 10KΩ between 6th, 7th pins. The obtained output was verified from the 3rd pin.

4.1.1 555 Timer Circuit and its Output Obtained in Multisim

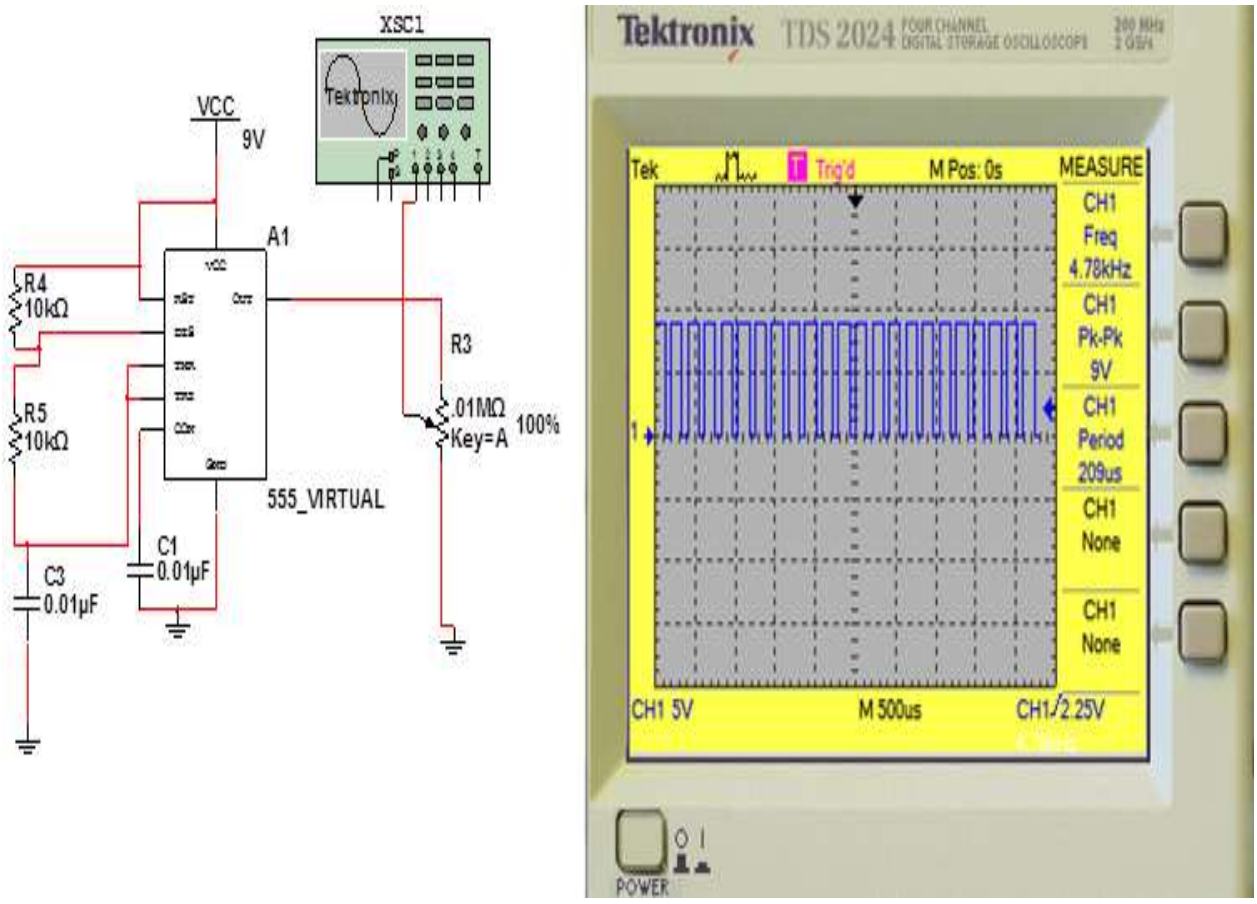

Figure 4.1: 555 Timer circuit output waveform

The micro-controller was used to control the number of pulses. Below mentioned circuit will help in understanding the controlled number of pulses. When the switch was on the pulses go on, and when the switch was off pulses stopped. Therefore, a programme was written for operating the switch (on/off). Then interfaced the microcontroller to the keypad to give the required number of pulses. The result was then sent to LCD display to see the given number of pulses.

4.1.2 Relay output in Multisim

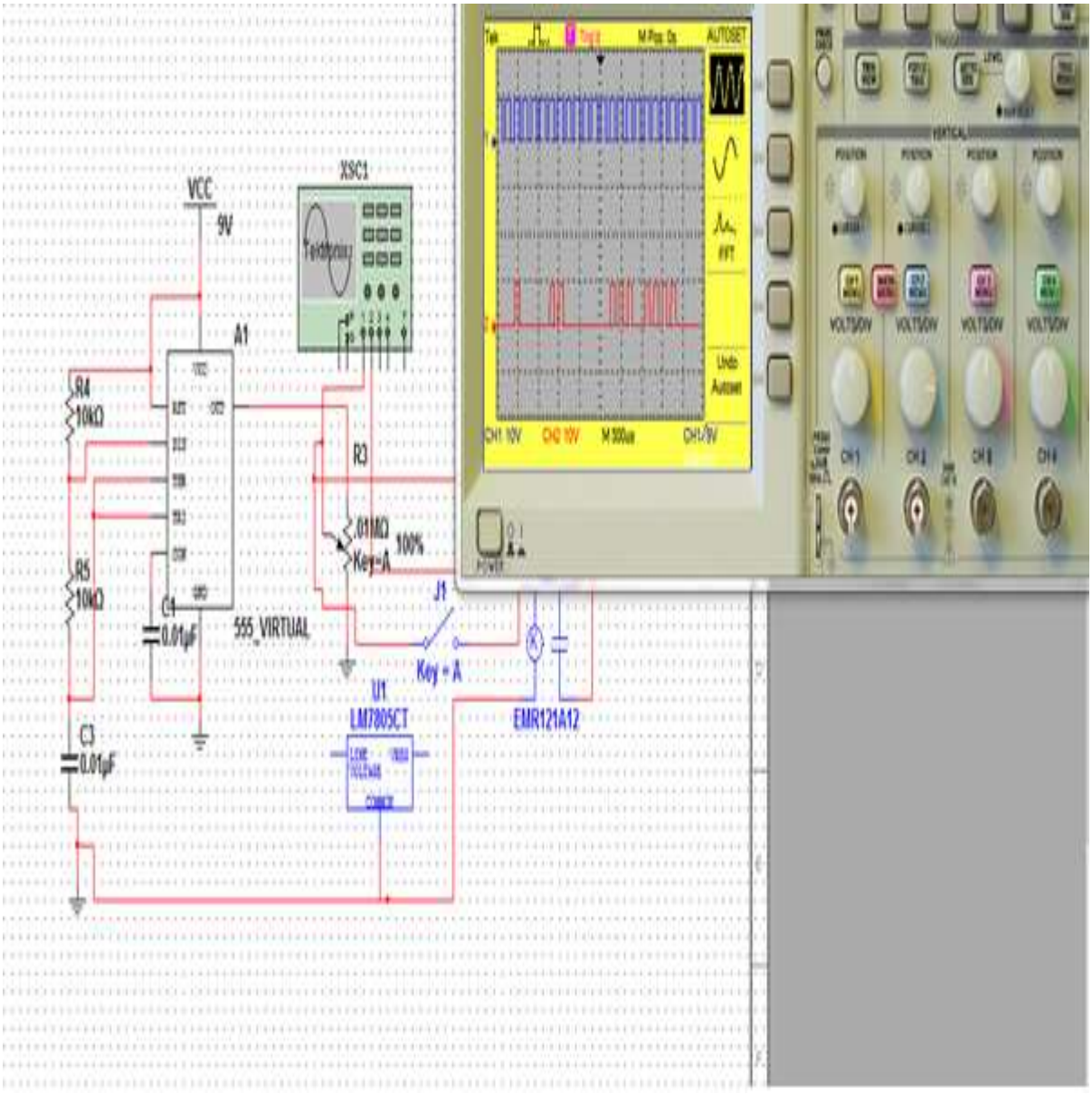

Figure 4.2: Relay Output Waveform

A micro-controller was used to control the number of pulses for operating the relay (on/off). Below mentioned programme helped in controlling the number of pulses through relay of 12V.

4.1.3 Microcontroller Programme for Controlling the Pulses at Pulse Timer Output:

The programme written in C++ language on keil (µ vision4, UN, USA) to make the output of the regulator readable by the Microcontroller (89C52) is shown in Table 4.1.

Table 4.1: The program in C++ to control the number of pulses

```
#include<reg51.h>
#define lcdport P2
sbit in = P0^0;
sbit out = P1^0;
sbit enter=P0^1;
sbit reset=P0^2;
sbitrs=P1^4;
sbit en=P1^6;
sbitrw=P1^5;
sbit R1=P3^0;
sbit R2=P3^1;
sbit R3=P3^2;
sbit R4=P3^3;
sbit c1=P3^4;
sbit c2=P3^5;
sbit c3=P3^6;
voidlcdcmd (char);
voidlcdint ();
voidlcddata (char);
voidlcdstring (char *);
voidlcdnumber (int);
void delay(unsigned int);
//void delaya(void);
charscan_key();
intget_number(void);
void Display(int);
void pulse();
int p=-1, key=-1, no=0;
void main()
{
//inti=0;
out=0;
lcdint();
lcdstring("welcome 2 keypad");
```

```
lcdcmd(0xc0);
lcdstring("press any key");P3= 0xf0;
delay(100);
lcdcmd(0x01);
lcdcmd(0x02);
while(1)
{
	while (key!='e')
	{
		key = scan_key();
		if ((key >= 0) && (key <= 9))
		{
			//lcdcmd(0x01);
			//lcdcmd(0x02);
			no=get_number();
		}
	}

	if (key=='e')
	{
		lcdcmd(0x01);
		lcdnumber(no);
		pulse();
//
//		if (in == 1)
//		{
//			if (i<(8*no))
//				{
//					out=1;
//					i++;
//				}
//			else
//				out=0;
//		}
//		else
//			out=0;

	}
}
}

charscan_key()
{
char key;
R1=0,R2=1,R3=1,R4=1;
if(c1==0){delay(100);key=1;return key;}
if(c2==0){delay(100);key=2;return key;}
if(c3==0){delay(100);key=3;return key;}

R1=1,R2=0,R3=1,R4=1;
```

```
if(c1==0){delay(100);key=4;return key;}
if(c2==0){delay(100);key=5;return key;}
if(c3==0){delay(100);key=6;return key;}

R1=1,R2=1,R3=0,R4=1;
if(c1==0){delay(100);key=7;return key;}
if(c2==0){delay(100);key=8;return key;}
if(c3==0){delay(100);key=9;return key;}

R1=1,R2=1,R3=1,R4=0;
if(c1==0){delay(100);key='r';return key;}
if(c2==0){delay(100);key=0;return key;}
if(c3==0){delay(100);key='e';return key;}

return -1;
}
intget_number()
{
        int j;
        for (j=0; j<10; j++)
        {
                if (key == j)
                {
                        no=no*10+j;
                        Display(key);
                }
        }
        return no;
}
void Display(int key)
{
        if (key == 1) { lcddata('1'); delay(20); }
        if (key == 2) { lcddata('2'); delay(20); }
        if (key == 3) { lcddata('3'); delay(20); }
        if (key == 4) { lcddata('4'); delay(20); }
        if (key == 5) { lcddata('5'); delay(20); }
        if (key == 6) { lcddata('6'); delay(20); }
        if (key == 7) { lcddata('7'); delay(20); }
        if (key == 8) { lcddata('8'); delay(20); }
        if (key == 9) { lcddata('9'); delay(20); }
        if (key == 0) { lcddata('0'); delay(20); }
}
void delay(unsigned int x)
{
unsignedinti,j;
for(i=0;i<x;i++)
for(j=0;j<500;j++);
 }
/*void delaya()
        {
```

```
                int k;
                for(k=0;k<10000;k++);
        }
*/



voidlcdint()
 {
lcdcmd(0x38);
delay(1);
lcdcmd(0x0F);
delay(1);
lcdcmd(0x01);
delay(1);
lcdcmd(0x06);
delay(1);
lcdcmd(0x0e);
delay(1);
 }
voidlcdcmd(char value)
{
lcdport = value;
rw=0;
rs=0;
en=1;
delay(1);
en=0;
}
voidlcdstring(char *P)
{
while(*P!='\0')
  {
lcddata(*P);
delay(10);
   P++;
  }
}
voidlcddata(char value)
{
lcdport = value;
rs=1;
rw=0;
en=1;
delay(1);
en=0;
}
```

```
voidlcdnumber (int value)
{
	intc,i;
	int d[10];
	c=value;
	for(i=0; i<10; i++)
	{
		d[i]=c%10;
		c=c/10;
		if(c==0) break;
	}

void pulse()
{
inti=0;
while(1)
	for(;i>=0;i--)
		lcddata((48+d[i]));
}

{
	delay(10);
	if (in == 1)
		{
			if (i<30)//(2*(no-(no/10))))
				{
					out=1;
					i++;
				}
			else
				out=0;
		}
	else
		out=0;
}
}
```

4.1.4 Circuit Diagram

For operating the micro-controller programme the compatible version of Proteus circuit design software was used. Below is the circuit design of Electroporator output. Each stage of the output was checked to confirm the desired output.

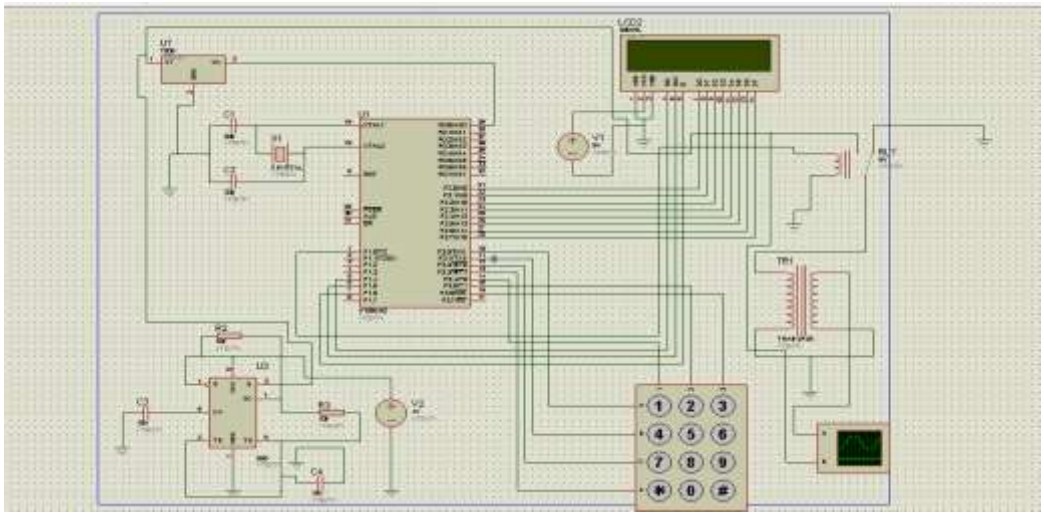

Figure 4.3: Circuit Diagram of Electroporation System in Proteus

4.1.5 555 output in Proteus

555 Timer circuit was connected in Proteus and observed its pulse output waveform.

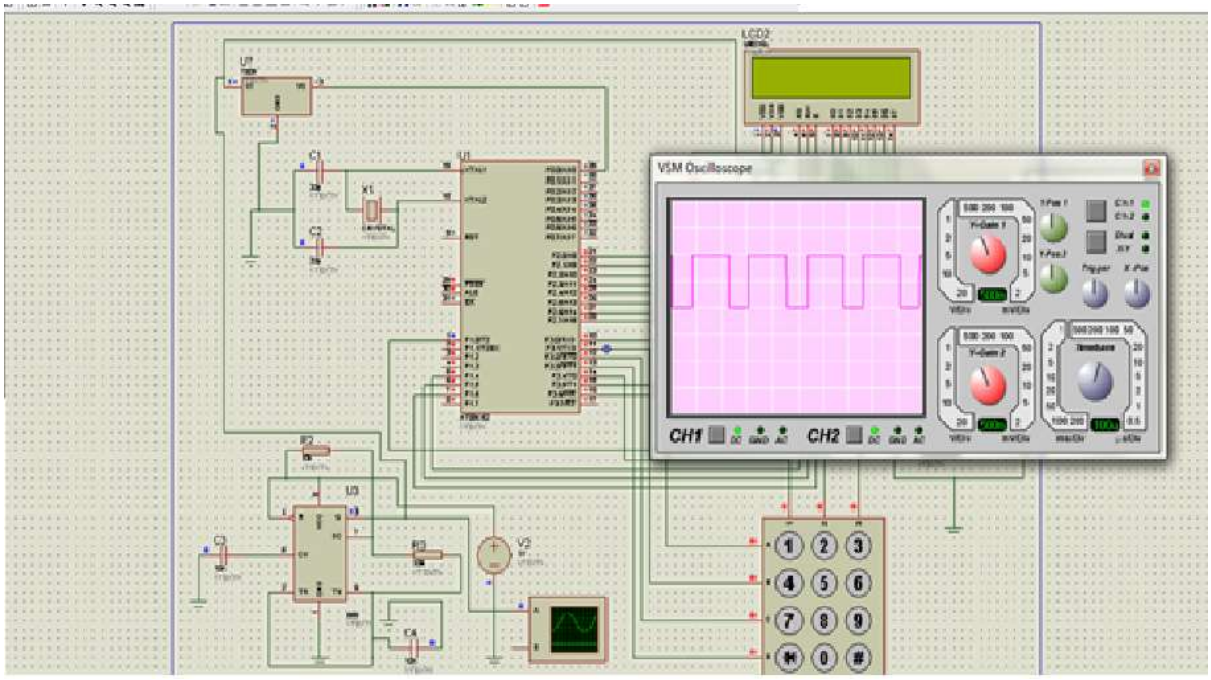

Figure 4.4: 555 timer output in Proteus

4.1.6 7805 output in Proteus

555 pulse output was given directly to 7805 regulator to step down the pulsating voltage to below 5V for micro-controller compatibility.

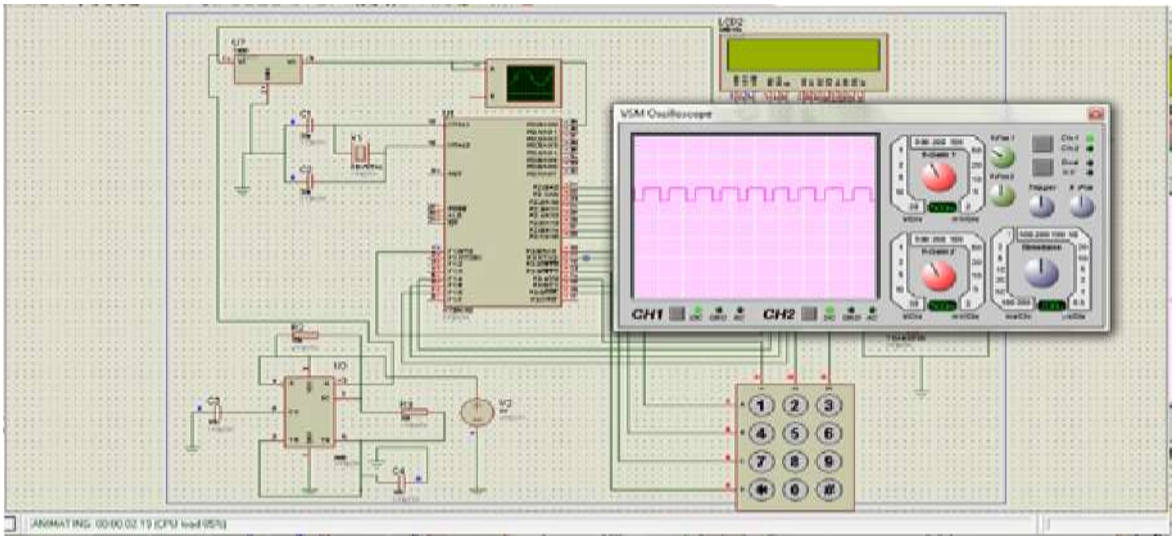

Figure 4.5: Regulator (7805) output in Proteus

4.1.7 Microcontroller output on Proteus

When the relay switch was in ON-mode by the microcontroller, continuous pulses of 5V range were obtained.

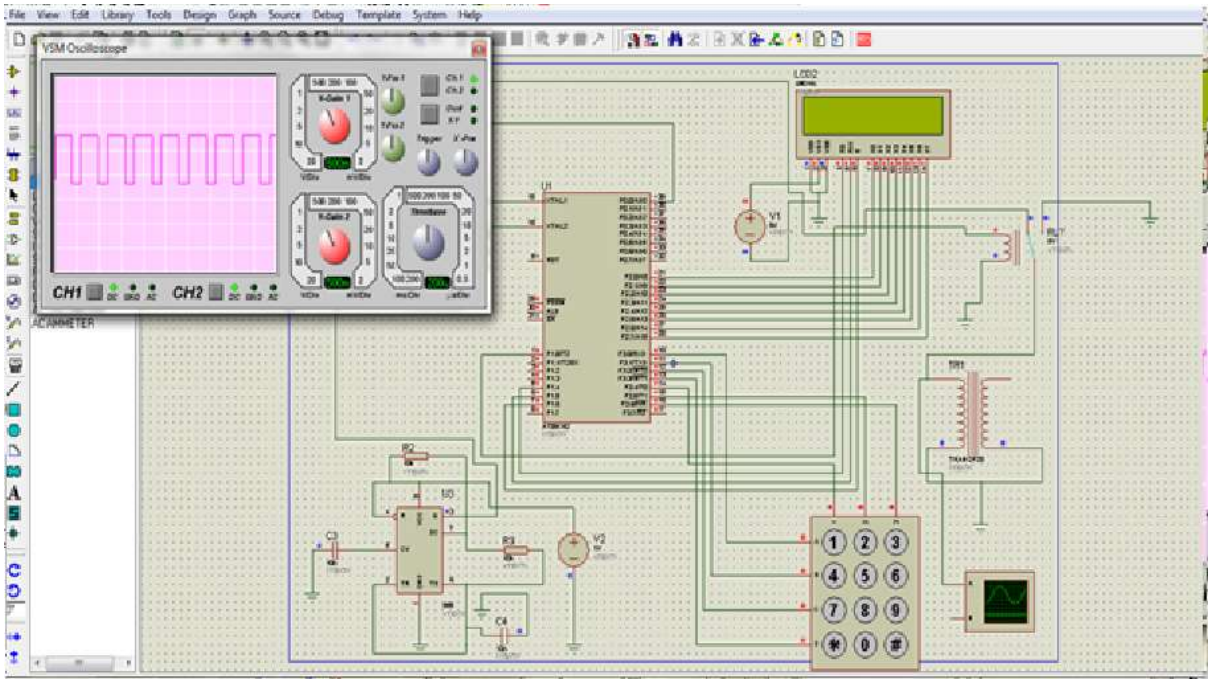

Figure 4.6: Microcontroller output in Proteus when relay is in on mode

When the relay switch was in OFF-mode by the microcontroller, transformer output became Null.

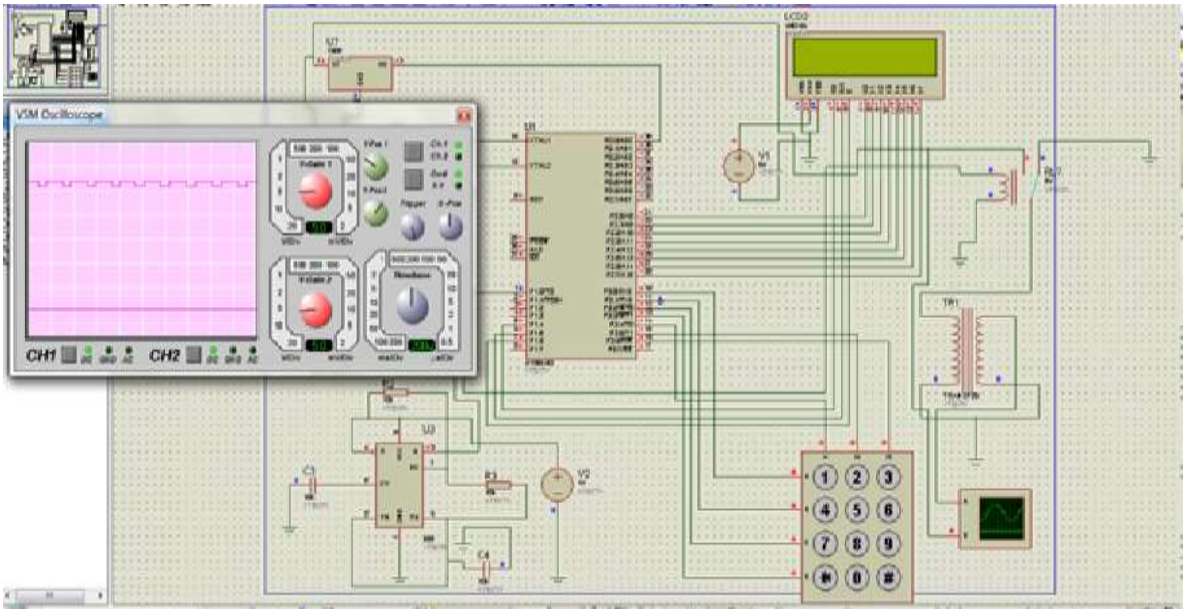

Figure 4.7: Microcontroller output in Proteus when relay is in off mode

4.1.8 Distinguishing the 555 Output and the Transformer Output in Proteus

When the output waveforms of both the regulator and transformer were observed, it was found that the transformer produced a waveform of 700V.

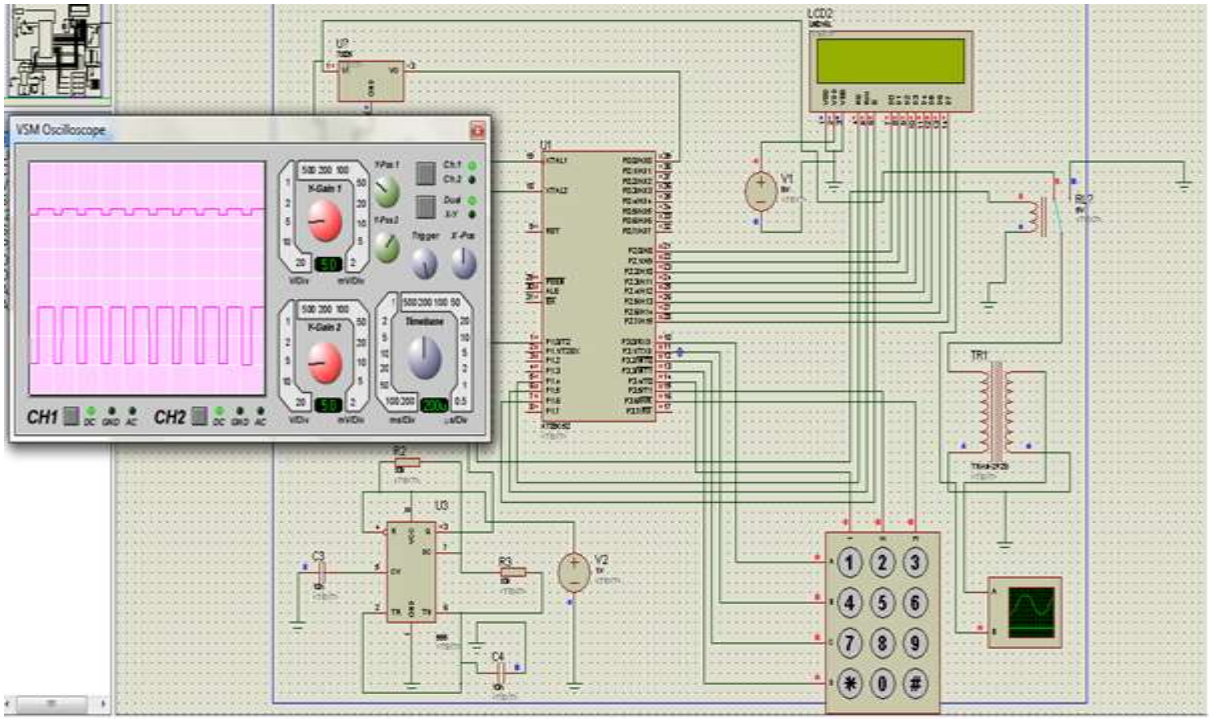

Figure 4.8: Transformer output in Proteus

4.2 EXPERIMENTAL RESULTS

4.2.1 555 Timer Output

555 Timer circuit was connected on breadboard and its output waveform was found to be pulsating DC of voltage between 8 to 9V.

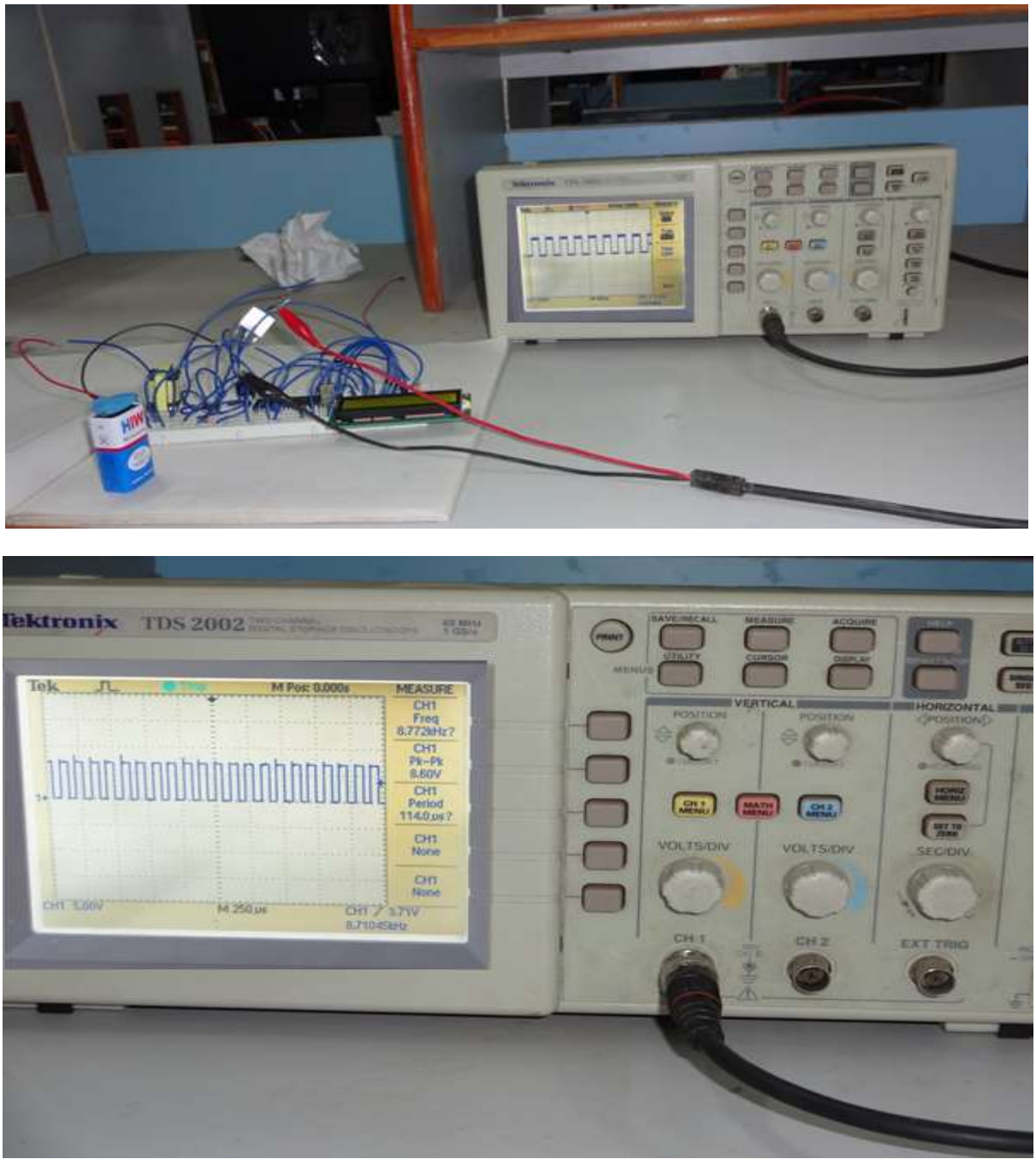

Figure 4.9: Experimental 555 Timer Output

4.2.2 Regulator output

555 timer output was connected to 7805 regulator input to observe the regulated output. It was found to be a pulse waveform 4-5V range

Figure 4.10: Experimental 7805 Regulator Output

4.2.3 Microcontroller relay output (number of pulses given=20)

Regulator output was given to the relay circuit to control the number of pulses and gave 20 pulses through programming. Train of pulses of 4V with 20 number of pulses per second was obtained.

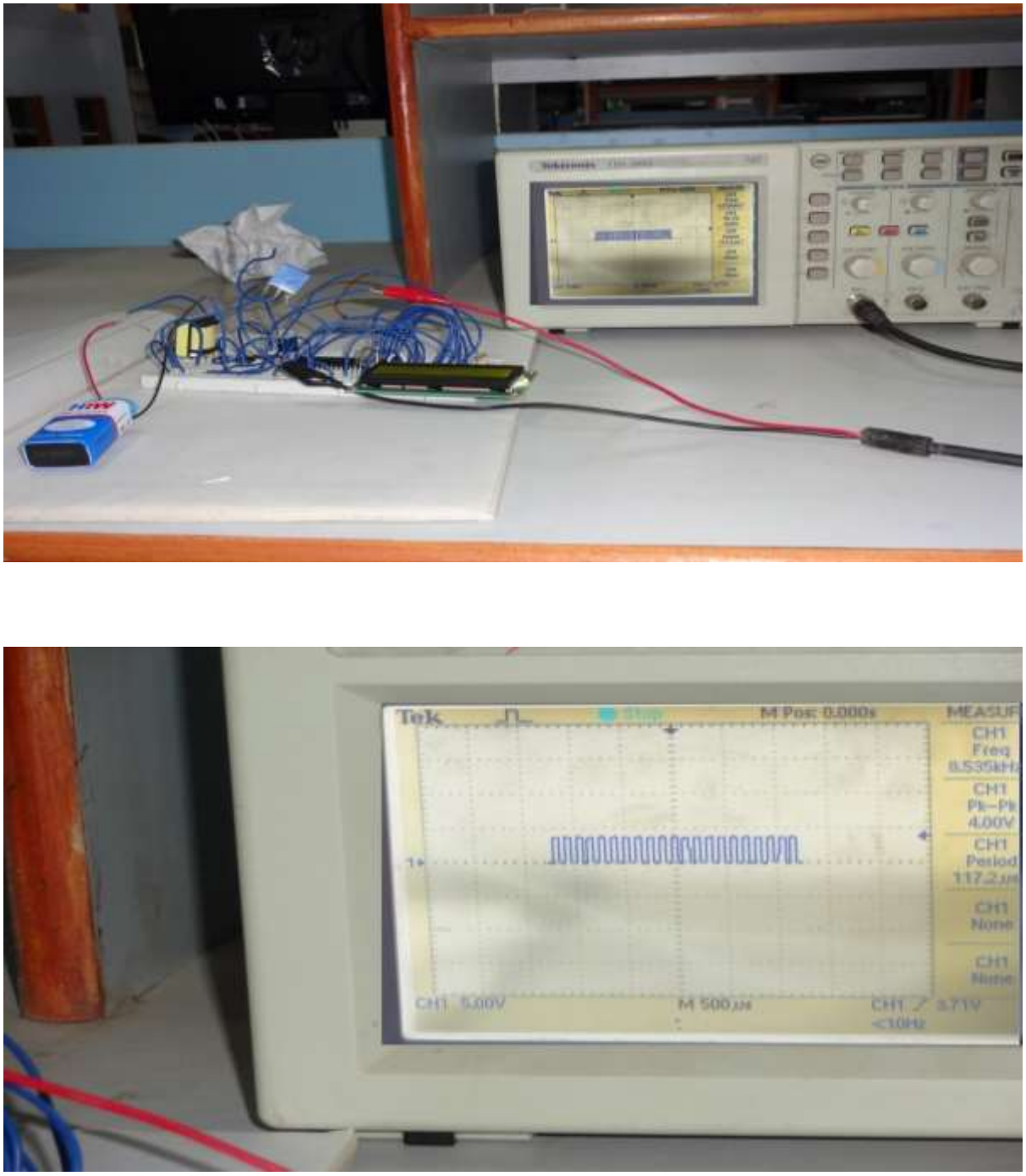

Figure 4.11: Experimental Microcontroller Output

4.2.4 Microcontroller Transformer Output (number of pulses given=3)

The relay output pulse was given to the output of the transformer circuit to control the number of pulses that produced 3 pulses through programming.

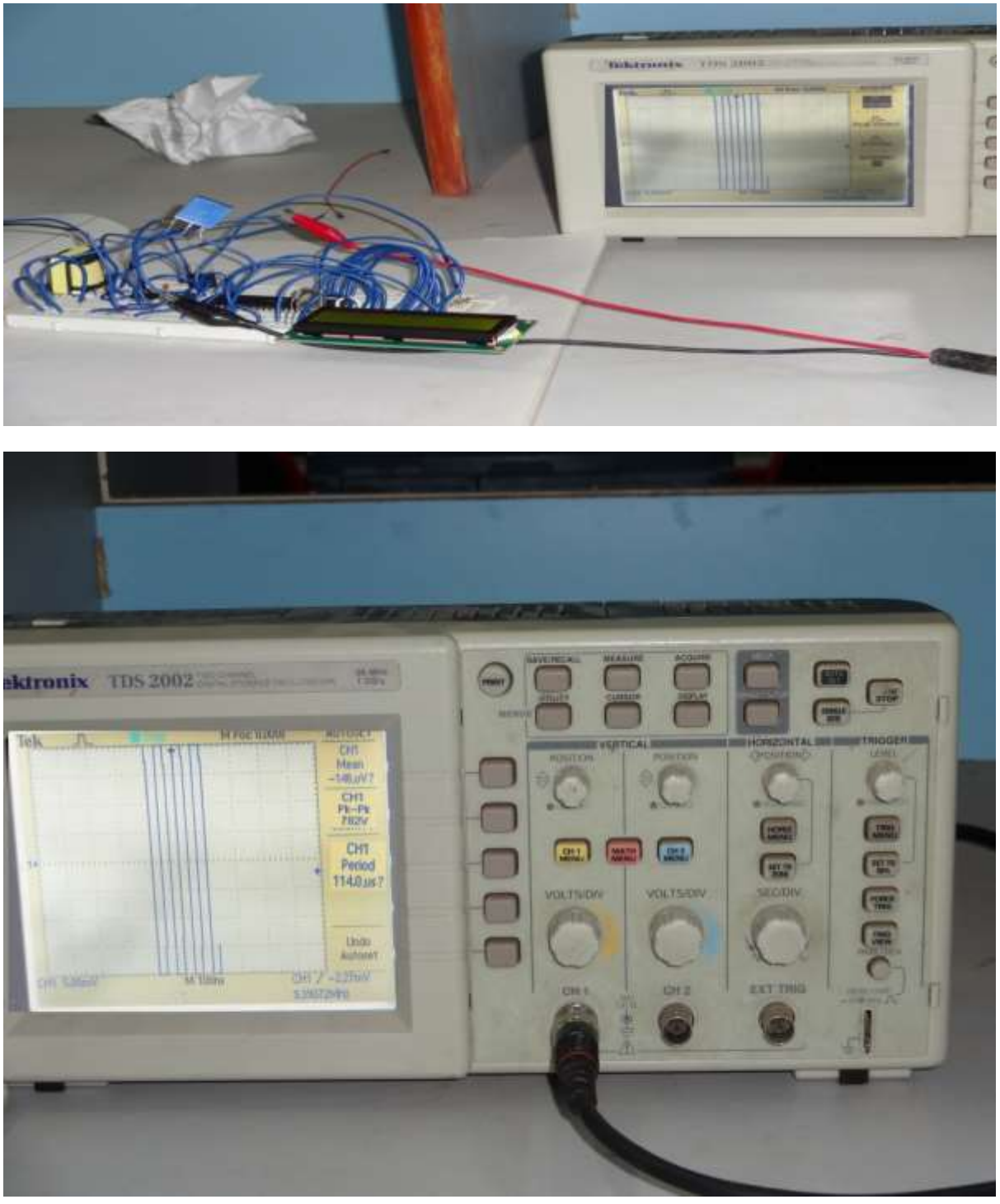

Figure 4.12: Experimental Transformer Output

4.3 PARAMETERS ACHIEVED FOR ELECTROPORATION SYSTEM

Table 4.2: Obtained Parameters of Proposed Electroporator System

Parameter	**Value**
Pulse voltage	782V
Pulse duration	50µs-1000ms
Number of pulses	1-10000
Pulse current	1-10mA

CHAPTER- 5

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CONCLUSION

5. CONCLUSION

In the current study, a low cost glass slide based Electroporator system was designed and fabricated successfully with a very small foot print. A high voltage generator based on a transformer was successfully integrated to get an instantaneous pulse of very high voltage up to 782 V that is essential for increase the conductivity of live cell membrane temporarily. At the same time a high precision pulse generator was embedded with the system that can generate a maximum of 20000 pulses per second (a pulse with drastically low duration of 50µs). DC to AC showed that the electroporation time and cell damage depends upon number of pulses and cell size. The potentiometor part successfully changed the voltage between 300 to 700 V while the pulse duration varied from 50 µs to 1000 ms with high precision. This confirmed that the electropoartor system can be utilized to transfect a wide array of cell lineages with molecules of different physical properties and sizes. Since transfection capability of different cells vary to a great extent (i.e. fibroblast are easy to transfect while stem cells are highly resistant; mammalian cells are more resistant to be transfected compared to bacterial cells), the in house developed Electroporator system has the potential to work for different cell lines.

The future work can be extended to improvise the device for obtaining an output voltage up to 1100 V for stem cell transfection. In addition before its commercial application, the device needs to be characterized for its capability of live cell transfections *in vitro*.

CHAPTER- 6

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